Remarks/Arguments

Withdrawal of the previous rejection under 35 USC § 102(b) over Uehara et al. is gratefully acknowledged.

Claims 33-34, 36-37, 43-50, 59-65, 71-78, and 87-120 are pending in the application and stand rejected. Claims 33, 43, 59, 63, 71, 90, 102, 106, and 109, are amended.

Rejection Under 35 U.S.C. § 112, first paragraph – written description

Claims 33-34, 36-37, 43-50, 59-65, 71-78 and 87-120 stand rejected under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. The Examiner has asserted that the limitation that "the level of the enzyme 'activity' in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of the direct inhibitor or activator of the enzyme" appears to require that the target enzyme be enzymatically active in the cell in the presence of the inhibitor or activator. incorporates new matter. The Examiner has also asserted that the requirement that the POI be enzymatically active when bound to an inhibitor constitutes new matter.

The specification makes clear that the invention relies on controlling the level of a POI in a test cell. The POI is expressed at a level sufficient to produce an observable phenotypic characteristic that is responsive to the amount of the POI and to the specific activity (which can be modulated by activators or inhibitors) of the POI. Further, the greater the amount of the POI, the greater the response observed in the test cell. Maintaining the amount of the POI allows any change in the responsive phenotypic characteristic to be attributed to inhibition or activation of the POI.

That is the point of the abovementioned limitation – that any observed modulation of a selected phenotype that is linked to the presence of the POI is due to inhibition or activation of the POI, rather than due to a change in the amount of the POI. The word 'activity' was inserted into the limitation after 'enzyme' in response to the Examiner's assertion that it was "unclear if the enzyme (POI) must be maintained in an active form in the cell when in the presence of (and bound to) the inhibitor." In his remarks accompanying the amendment, the Applicant pointed out that the enzyme activity (in the absence of inhibitor or activator) in the test cell should be maintained such that the test cell is capable of exhibiting the same

phenotypic response once a direct activator or inhibitor is removed, as prior to the addition of the inhibitor or activator.

With regard to new matter, the rejection is now moot. Regarding the Examiner's assertion that the claim that was pending prior to this Amendment constituted new matter, it seems to the Applicant that the claims did not actually require that the target enzyme (POI) be enzymatically active in the presence of the inhibitor or activator. The claims literally stated that the level of the enzyme activity is maintained such that the cell remains capable of exhibiting the phenotypic response once the inhibitor or activator is removed. Maintaining the enzyme activity means that the responsive phenotype can still be modulated by subsequent exposure to the same or other inhibitor or activator. The claims only required that the POI be enzymatically active before the inhibitor is added and after the inhibitor is removed. Moreover, the Examiner's interpretation would be contrary to any reasonable definition of an inhibitor.

Applicants amendments are also mindful of Examiner's previous indefiniteness rejection (4/20/04 Office Action) with regard to whether the enzyme (POI) must be maintained in an active form. The claims now make clear that the test cell remains capable of expressing the responsive phenotype that results from expression of the POI at essentially the same level (*i.e.*, the level or amount of the POI in the test cell is substantially the same prior to addition of the test compound, in the presence of the test compound, and after removal of the test compound).

Rejection Under 35 U.S.C. § 112, first paragraph - enablement

The rejection of Claims 33-34, 36-37, 43-50, 59-65, 71-78 and 87-120 for lack of enablement is respectfully traversed.

The Examiner has suggested that the Applicant's response with respect to Hsiao et al. was more appropriate to a showing that Hsiao's work is patentably distinct from that of the applicant. To the contrary, the Applicant was arguing the inappropriateness of applying the Hsiao reference to suggest the unpredictability of attempting to identify inhibitors or activators of an enzyme based on the claimed method is inappropriate. Hsiao is an inappropriate reference because it discloses a method that is different than what is disclosed and claimed by the Applicant.

One of ordinary skill in the art at the time the application was first filed would understand the differences between the purposes of Hsiao's work and the groundbreaking purpose of Applicant's invention. Hsiao's work was directed to understanding whether genetic changes in a cell could combine with environmental effects to result in cell transformation. In contrast, the instant invention "provides a rapid yet powerful screening system for the discovery and identification of both activators and inhibitors of proteins." (Specification, p. 4, ll. 18 - 20; '281 patent, col. 2, ll. 44-46). Hsiao provided no means for determining whether any chemical agent directly or specifically interacted with any cell component, and there was no suggestion that such was the case. One of ordinary skill in the art would also have recognized the elements of Applicant's disclosed method, which are absent from Hsiao, as critical to the workings of the invention. The specification even discloses how to use Hsiao's Rat-6 cells, remarking that what is sought is an "increase in the phenotypic change exhibited by the cell which becomes greater with increasing expression of the POI." (Specification, p. 12, ll. 22 - 24; '281 patent, col. 5, l. 66 to col. 6, l. 20) To justify a claim of unpredictability, the Examiner is mixing apples and oranges because Hsiao's method is completely different from the claimed method.

The Examiner states that Hsiao makes it clear that if chemical agents such as TPA or teleocidin are tested against cells expressing $p21^{ras}$, a greater phenotypic effect would be observed on a test cell expressing $p21^{ras}$ compared with a control cell. Such is anything but clear from Hsiao's results. Hsiao discloses that, in the absence of $p21^{ras}$ transfection, TPA did not induce transformed foci. Hsiao also discloses that Rat 1 cells transfected with $p21^{ras}$ developed essentially the same number of foci regardless of the presence or absence of TPA (Table 1). Further, where the effect purported by the Examiner to be clear is observed (Table 1, Rat 6 cells), the "phenotypic effect" is merely focus formation observed in cultures of $p21^{ras}$ transfected cells carrying widely varying levels of $p21^{ras}$ (see, *e.g.*, Fig. 2). Nothing in Hsiao even suggests the concept of the claimed responsive phenotypic change, much less a graded cellular response (recited in Claims 62, 63-65, 71-78, 105, 106-108 and 109-116.)

What Hsiao does not teach is clearly taught by the Applicant's specification. In particular, the specification teaches that the phenotypic change that should be observed is one that is responsive to the POI. For example, as noted above, the specification directs the

practitioner to look for an increase in the phenotypic change exhibited by the cell which becomes greater with increasing expression of the POI. The specification also teaches that the practitioner make use of the phenotypic response observed in the presence of a known activator or inhibitor. For example, the specification teaches that if one were interested in screening for a protein kinase C (PKC) inhibitor, cell lines would be used which grow well in soft agar (as a result of their overproduction of any form of PKC) and yet show an enhancement of their growth when compounds which are known to stimulate PKC are added to the growth medium. Appropriate control cells, would not exhibit these characteristics. (Specification, p. 22, ll. 17 - 24; '281 patent, col. 10, ll. 5-12).

In short, Hsiao makes no pretense that TPA is an activator of $p21^{ras}$ and one of skill in the art would not assume so. One of skill in the art reading Applicant's specification would understand that Hsiao's method does not accomplished the claimed result because Hsiao's experiments provide no useful correlation between the presence of $p21^{ras}$ in a cell in unknown amounts and focus formation in the presence (or absence) of TPA. Hsiao's results cannot be an indication that the Applicant's invention is unpredictable, because Applicant teaches a concept that is drastically different from anything that might be imagined on the basis of Hsiao.

The Examiner has asserted that the Ledwith et al., Mol Cell. Biol. Vol. 10, 1990, ps. 1545-1555, ("Ledwith") shows a hypothetical example of a putative false positive result if one were to use Applicant's method. Ledwith utilizes plasmid constructs harboring a c-fos fragment in the sense and anti-sense orientations and whose transcription is driven by the mouse mammary tumor virus promoter, which is dexamethasone inducible (See p. 1545, right hand side, Materials and Methods, first paragraph, through 1546, left hand side, first and second sentences; and p.1546, right hand side, Results Section, first para). Ledwith introduces these constructs into NIH 3T3 cells that have already been transformed with c-Ha-*ras*, thus creating $p21^{ras}$ overproducing cells harboring either sense or anti sense c-*fos* constructs as well. Thus, the antisense construct should generate antisense c-fos RNA which will reduce the level of functional fos mRNA and therefore also reduce the endogenous levels of c-fos protein in the cell relative to control cells (Fig. 8 legend, p1552-53; see also p 1551, Discussion section, left hand side, second full paragraph).

In particular, the Examiner has asserted that Figure 4 of Ledwith shows that cells transformed with $p21^{ras}$ and exhibiting a transformed phenotype resulting from $p21^{ras}$ expression exhibited a graded cellular response (with regard to the transformed phenotype) to increasing or decreasing levels of c-*fos*. (Office Action, bottom of page 5). Applicant most respectfully disagrees as this is *not* a graded cellular response as defined by Applicant. Rather, in the specification Applicant explicitly defines the concept of the graded cellular response as one which is driven by the overproduction of the POI, and not as a result of alterations in the levels of a second protein other than the POI (in this case c-*fos*).

Next, the Examiner has also asserted that the Ledwith paper hypothetically shows that if the skilled artisan used Applicant's method to attempt to identify an inhbitior of $p21^{ras}$ and the test compound actually directly bound to and inhibited c-fos, the skilled artisan would erroneously identify the compound as an inhibitor of $p21^{ras}$. Applicant most respectfully traverses this argument for multiple reasons. First, and most importantly, Ledwith concedes that their antisense method is NOT capable of fully reverting the tranformed phenotype that results from c-Ha-ras overproduction. The authors concede this fact in several places (since it is known to those skilled in the art to be true anyway) irrespective of how high is the dose of dexamethasone that is used and regardless of the duration of exposure. See, for example, p. 1545, Abstract, lines 6-8; p. 1551, right hand side Discussion section, first paragraph: "However, antisense-fos RNA caused a *partial* reversion in several of the transformed phenotypes of EJ cells...(emphasis added). See also, p. 1554, last paragraph: "In conclusion, inhibition of c-fos gene expression by antisense-fos RNA causes a *partial* reversion of the uncontrolled growth and tumorigenic phenotypes induced by the EJ c-Ha-ras gene in NIH 3T3 cells. (emphasis added). In putting forth its argument that a skilled investigator would erroneously choose a compound exhibiting the pharmacological properties of dexamethasone in this highly unusual assay system (that would not be designed by the skilled investigator in the first place to search for $p21^{ras}$ inhibitors according to Applicant's teachings) the Patent Office has not explained why the skilled investigator would *ever* choose a compound that only *partially* reverts a properly selected phenotypic response (i.e. one selected according to Applicant's method, which of course has also not occurred in Ledwith from the outset). It is well-known to the skilled investigator that in performing a screening method numerous

compounds (usually tens or hundreds of thousands) will be tested on a given cell type, and will be rank ordered according to those with the highest degree of activity (potency) in the assay. Why would the skilled investigator arbitrarily choose a compound that only partially modulates a selected phenotypic response which, according to the teachings of Applicant's method, is fully driven by the activity of the (true) POI? This makes no sense, and Applicant asserts that the Examiner is placing an excessively undue burden on the Applicant for reasons unknown to him. As shown in Table 3 of Applicant's specification, it is clearly indicated that the compounds so tested, at selected concentrations, are capable of fully (not partially) reverting the properly chosen resposive phenotypic characteristic driven by the overproduction of PKC ß1 (See Col. 20 line 60, and Col. 21, lines 6-7, of the '281 patent).

Moreover, Applicant at no time has ever asserted that false positive results might not occur from time to time, as is the case with all known assay systems. But, a method need not be infallible to be enabled. A claim is not invalid simply it encompasses some inoperative embodiments. *Atlas Powder Co. v. E. I. duPont de Nemours & Co.*, 750 F.2d 1569, 1576, 224 U.S.P.Q. 409, 414 (Fed. Cir. 1984). It is possible to argue that process claims encompass inoperative embodiments on the premise of unrealistic or vague assumptions, but that is not a valid basis for rejection. *In re Geerdes*, 491 F.2d 1260, 1265, 180 U.S.P.Q. 789, 793 (CCPA 1974). The PTO has not provided even one single example of a documented false positive result using Applicant's method. In fact, Applicant represents that Applicant is unaware even today in the medical or patent literature of any documented example of a false positive result having occurred, despite the fact that Applicant's method has been licensed and is presently in use in 37 of the top 50 research based pharmaceutical concerns world-wide. Applicant can however, provide authoritative publications demonstrating that *in vitro* receptor binding assays that the Examiner has suggested on past occasions would be necessary to validate the results of Applicant's screen, can in fact lead to erroneous results and that Applicant's cell-based systems have indeed been utilized to obtain the correct results in such circumstances. Applicant would be pleased to provide exemplary references at the Examiner's request.

Applicant respectfully points out the following additional reasons why the Ledwith manuscript cannot suggest lack of enablement:

1) As the Examiner is aware, dexamethasone does not bind to and inhibit c-fos. Rather, it induces expression of anti-sense c-fos through the MMTV promoter present in the construct. Thus, the cells are not being treated with the anti-sense RNA at all; pM(84)fos^{S} and pM(84) fos^{AS} constructs have been cloned into the cell lines and used to induce sense or anti-sense fos expression, respectively. There is no evidence that a true chemical agent that binds to and inhibits fos would work to the same degree of efficacy. Furthermore, even this elegant, complicated approach still failed to fully revert the p21^{ras} transformed phenotype, as discussed above.

2) The Examiner has asserted that the Ledwith paper suggests that a compound that scores positively in the Ledwith assay would require further work-up to verify that it bound to c-fos rather than p21^{ras}. However, since Ledwith has not defined a responsive change in a phenotypic characteristic according to the teachings of Applicant's method for either p21^{ras} or c-fos in these experiments (nor was it the authors intention to do so), no conclusions may be drawn regarding this point.

3) The Examiner has stated that "since many eukaryotic enzymes are involved in complex signal transduction pathways in cells, it must be considered that attempting to identify direct inhibitors or activators of any given enzyme by the claimed method would be unpredictable without further experimentation to actually identify whether the test compound actually directly binds to the putative target POI."

Applicant respectfully but vigorously disagrees with the Examiner on this fundamental, most essential point of Applicant's invention. Indeed, the Ledwith paper provides yet additional support as to why Applicant's method is so highly specific with respect to the POI. Even by using a complicated method to very precisely knock out in an inducible manner the expression of an important downstream signaling protein of p21^{ras}, *the authors are unable to completely revert the p21^{ras} –transformed phenotype, as they readily concede (see above).* This is precisely because as the Examiner has pointed out, many eukaryotic enzymes are involved in complex signal transduction pathways in cells, and thus no single downstream protein is solely responsible for the effects of any one of said enzymes. However, when one uses Applicant's method and overproduces a given POI, the cellular effects of said POI are greatly amplified, *including its ability to signal down multiple*

pathways simultaneously. This is why Ledwith is unable to fully revert the EJ (ras transformed) cell lines with an antisense fos construct, because skilled investigators know, as the Examiner has pointed out, that eukaryotic proteins are involved in complex signal transduction pathways in cells, and no one single pathway mediates the enhanced cellular functioning of an overproduced POI such as $p21^{ras}$. Thus, no inhibitor or activator of a protein other than the target POI ($p21^{ras}$ in this case) will have the same degree of effect on the defined phenotypic response as will a compound that interacts with (binds to) the POI in order to exert its effect. This is one of the key reasons why Ledwith fails to fully revert the ras-transformed phenotype in EJ cells despite the use of the anti-sense fos construct approach. If c-fos did indeed mediate all of the cellular effects of $p21^{ras}$, than a genetically engineered anti-sense fos construct should have completely reverted the transformed phenotype of the EJ (ras-transformed) cell lines. But despite their best efforts, the authors concede that they are unable to fully revert the $p21^{ras}$ transformed phenotype, and further acknowledge that other pathways contribute to the phenotype. The authors' results emphasize why the Ledwith case is unlikely to be a false positive result in Applicant's method. Applicant regards this as highly unlikely, and reiterates that no party, including the USPTO, has ever shown a definitive example of the identification of a true false positive result using Applicant's method.

In contrast, Applicant has demonstrated the effectiveness of the method. The Applicant has previously drawn the Examiner's attention to the fact that, as provided by a working example, tamoxifen, previously known only as an anti-estrogen, was identified to be a PKC inhibitor in a cellular system. The Examiner questioned this statement (citing O'Brian et al., 1985, Cancer Res. 45:2462-65, as disclosed by the Applicant), suggesting that tamoxifen was previously known to be a PKC inhibitor *in vitro*. A careful reading of the article will show only that PKC inhibition was observed when tamoxifen was added to an assay system containing other components necessary for PKC stimulation, not that tamoxifen is an inhibitor of PKC. Binding of tamoxifen to PKC *in vitro* was confirmed *after* the Applicant's disclosed work (see, Exhibit A, O'Brian, Housey et al., "Specific and Direct Binding Of Protein Kinase C to an Immobilized Tamoxifen Analogue", 1988, Cancer Res.

48:3626-29, previously cited by Applicant and discussed in the Amendment filed May 19, 2003). In that article, the authors state:

> Kinetic studies provide evidence that the inhibition of PKC by tamoxifen involves *nonspecific* interactions between the antiestrogen and the lipid cofactor (13-15). However, the possibility that tamoxifen also has direct interactions with PKC has not been addressed prior to this report. Here we show that PKC *binds to* an immobilized analogue of tamoxifen *directly and specifically*.

O'Brian et al. (Exhibit A) at p. 3626, paragraph spanning cols. 1 and 2 (emphasis added).

In summary, the specification discloses and supports with working examples, a method that discriminates chemical agents that directly interact with a POI. In contrast, when fully considered, the references cited by the Examiner provide an unrealistic basis for speculation that the claims are not enabled. For the reasons set forth, Applicant contends that the instant claims comply with the requirement for enablement. Withdrawal of the outstanding rejection is respectfully requested.

Rejection Under 35 U.S.C. § 112, first paragraph – written description

Claims 33-34, 36-37, 43-50, 59-65, 71-78, and 87-120 stand rejected under 35 USC § 112, 1st paragraph, as failing to comply with the written description requirement. The Examiner continues to assert that the claims contain subject matter that is not described in the specification in a way that conveys possession of the claimed invention.

As set forth above, Applicant asserts that a method that discriminates chemical agents that directly interact with a POI is clearly disclosed in the specification. The specification teaches the elements that are critical to that discrimination and provides working examples that incorporate those elements. Most importantly, as pointed out above with regard to enablement, the specification clearly sets forth the features that were lacking in the art, which make cell based assays for identification of direct inhibitors or activators of an enzyme a reality.

In view of the arguments in this amendment and of record, the Applicant asserts that the claimed invention complies with the written description and respectfully requests reconsideration and withdrawal of the instant rejection

Rejection Under 35 U.S.C. § 112, second paragraph - indefiniteness

Claims 33-34, 36-37, 43-50, 59-65, 71-78, and 87-120 were rejected under 35 USC § 112, second paragraph, as indefinite for recitation of maintenance of enzyme activity in the presence of an inhibitor of the enzyme.

The rejection is made moot by the instant claim amendments. Withdrawal is respectfully requested.

Conclusion

It is believed that this amendment is fully responsive to the Examiner's rejections. Applicant feels that all references cited by the Examiner have been distinguished from the claimed subject matter. Should the Examiner find another reference which allegedly describes the invention and suggests that enablement is lacking, it is requested that the Examiner please contact the undersigned to arrange an interview so as to expedite prosecution.

In view of the foregoing amendments and remarks, it is firmly believed that the subject claims are in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

KENYON & KENYON

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By: _____
Lawrence P. Casson
Reg. No. 46,606

One Broadway
New York, NY 10004
Telephone: (212) 425-7200
Facsimile: (212) 425-5288